INSTRUMENTATION AND TECHNIQUES FOR MEASURING THE QUALITY OF INSECT VISION WITH THE ELECTRORETINOGRAM

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CONTENTS

ILLUSTRATIONS

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INSTRUMENTATION AND TECHNIQUES FOR MEASURING THE QUALITY OF INSECT VISION WITH THE ELECTRORETINOGRAM

By Herndon R. Agee[1]

ABSTRACT

An inexpensive and functional system for making electroretinograms was designed and constructed. The system, which features an insect-vision analyzer, is used to measure the visual sensitivity of insects (a measure of quality of an insect). The apparatus and electrophysiological techniques required to construct and use the system are described in sufficient detail for application by inexperienced persons. The system has immediate application in the quality-control sections of insect mass-rearing programs and presently is in use at three facilities. **KEYWORDS: electrophysiological techniques, electroretinogram, insect visual sensitivity, instrumentation, quality control.**

INTRODUCTION

Agricultural Research Service (ARS) and other institutions devote considerable effort to the production of insects in laboratories and factories for use on research and pest-control programs. If such research is to be valid, it is essential that laboratory-reared insects be like wild insects. Released into wild populations for control purposes, laboratory-reared insects must be able to compete successfully. But all too often these laboratory-reared insects do not behave or look like field insects or live as long (2, 3, 5–7).[2] The problem facing the researcher is early detection of behavioral, physiological, and other abnormalities in laboratory insect colonies.

It is reasonable to expect that diet, rearing procedures, genetic filtering, and other factors can contribute to the decline in visual sensitivity of a culture. In unpublished preliminary tests the author and cooperators found that the apple maggot fly, *Rhagoletis pomonella* (Walsh); the boll weevil, *Anthonomus grandis* Boheman; and the tsetse fly, *Glossina palpalis palpalis* (Robineau-Desvoidy), had lower visual sensitivities when cultured on nonhost food sources. Most of these insects are active fliers; therefore, vision is important to the life cycle of the adult in dispersing, locating mates, and finding hosts.

The author used the electroretinogram (ERG) to measure the visual sensitivity of several species of insects reared at various locations and compared their sensitivity to that of field specimens. Agee and Park (*2*) found that the visual sensitivity of laboratory-reared insects in many instances was less than that of field insects of the same age. Some samples had normal sensitivity, but considerable variability existed between species from different sources. These studies showed that a sample of 5 to 10 insects was sufficient to establish the visual sensitivity characteristics of a wild population or laboratory culture.

The ERG is an oscilloscopic display of the voltage changes that occur in the insect eye

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[2] Italic numbers in parentheses refer to items in "Literature Cited" preceding the appendixes.

when it is stimulated with flashes of light. The intensity of the electrical charge or response is directly related to the receptor sensitivity and the intensity of the stimulus.

The compound eye of most insects is composed of many light-receptor units called ommatidia. The photoreceptor cells in these ommatidia generate electrical potentials when they receive light stimuli and are the first-order level (detector) of the visual information aquisition process (fig. 1). At the second-order level, nerves from other visual photoreceptors synapse, or connect, to other cells to integrate, inhibit, or potentiate the passage of information on light reception from the ommatidial unit to the second- and third-order levels. The second-order level of information processing is farther and similarly processed at the third-order level in the "brain" of the insect to direct its behavior. Therefore, the visual information received by an insect at the first-order level is most important. The entire system for aquisition of visual information for the insect depends on the first-order cells, the photoreceptors, for the detection of visual information available in the insect's environment. Thus, an insect with low photoreceptor sensitivity would not be capable of detecting the same information as the insect with normal visual sensitivity. Information available but not perceived cannot influence the behavior of an organism. The less sensitive insect of a species would be a physiologically deficient insect (*4*).

The system described herein, which features the insect-vision analyzer, was developed for use as an early-warning system for "quality decline" in insect-rearing programs. The required instrumentation can be assembled with minimum expense and will provide the investigator with dependable, comparable measurements of the visual sensitivity of wild and laboratory-reared insects. This system is also useful for monitoring an insect colony on a batch, daily, or weekly basis and makes it easy to put a numerical value on insect quality (visual sensitivity). An excellent example of its usefulness has been demonstrated with the screwworm fly, *Cochliomyia hominivorax* (Coquerel) (*5*). Another example is the visual sensitivity studies presently being conducted at the International Atomic Energy Agency Laboratory in Seibersdorf, Austria, in cooperation with P. Vail and staff, on the tsetse fly

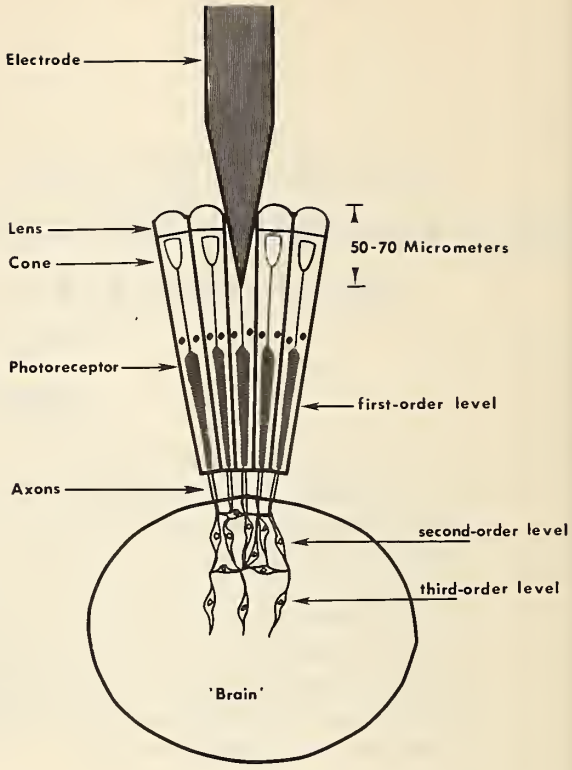

FIGURE 1.—Diagram of electrode position in compound eye of typical insect, showing relationship of electrode tip to receptor site or first-order level and second- and third-order levels of neural integration and inhibition in the "brain."

reared on horse blood (available through a membrane) and those reared on the blood of live guinea pigs. In the preliminary tests, five insects from each group were tested. Each insect was adapted to the dark for 20 to 25 minutes, and the differences in the visual sensitivity were very obvious for each group, with the insects that were fed on the blood of live animals being uniformly more sensitive. Only 4 to 5 hours, including the adaptation time, were required to run the ERG tests, but months of data accumulation would be required to measure differences in the two groups by using pupal weights or number of pupae produced.

The insect-vision analyzer was developed from more complex research instrumentation that was used to determine the visual sensitivity and spectral sensitivity of insects (*1*). The more complex system is composed of separate units that are adjustable over wide parameters. Since the insect-vision analyzer was

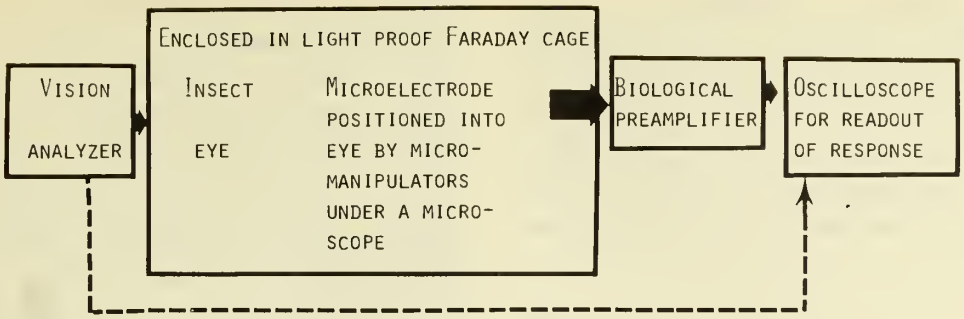

FIGURE 2.—Flow diagram of electroretinogram system.

designed and constructed to perform only the required functions for measuring the visual sensitivity of insects at one bandwidth with minimum equipment cost, much of the flexibility, including variable wavelengths and variable stimulus duration and rate, of the original system was omitted.

The insect-vision analyzer consists essentially of a filter-disk and light-chopper assembly, magnetic reed switch, motor, light pipe and filter assembly, lamp assembly, solar cell, and ammeter. A Faraday cage, micromanipulators, microscope, biological amplifier, and oscilloscope are also required to complete the system (fig. 2). The parts for this system cost about $60 in 1976 and require 24 to 30 hours to assemble, and the other components cost $2,700 to $3,000. A list of the instruments and materials required to record the ERG is given in appendix A.

The ERG techniques and instrumentation described have been tested extensively in this laboratory on the Caribbean fruit fly, *Anastrepha suspensa* (Loew), the boll weevil, and the bollworm moth, *Heliothis zea* (Boddie); in the ARS Screwworm Research Laboratory, Mission, Tex., on the screwworm fly; at the ARS Subtropical Horticulture Research Station, Miami, Fla., on the Caribbean fruit fly; and at the Insect and Pest Control Section, Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, Vienna, Austria, on the tsetse flies, *Glossina morsitans morsitans* Westwood, and *G. palpalis palpalis* (Robineau-Desvoidy).

The two goals of this paper are (1) to serve as a practical guide for those not experienced in the electrophysiology for assembling a simple functional system for recording electroretinograms of insects and (2) to provide guides for the use of the electroretinogram technique as a standard assay method in the quality-control section of an insect-rearing program. Since everyone's questions could not be anticipated, it is suggested that anyone encountering problems with the ERG system contact the author for assistance.

INSTRUMENTATION

Insect-Vision Analyzer

The insect-vision analyzer is assembled from relatively inexpensive components (fig. 3). (A list of materials required to construct the unit is given in appendix B.) Some fabrication

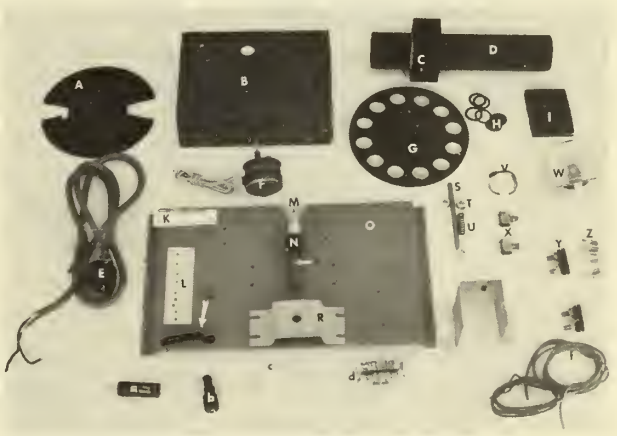

FIGURE 3.—Components of vision analyzer ready for assembly. A, Light chopper with magnet attached. B, Frame for light chopper and filter disk. C, Holder for 2- by 2-inch filters. D, Light pipe. E, Electrical connecter. F, Motor. G, Filter disk. H, Filters. I, Cover for filters. J, O-rings. K, Mounted magnetic reed switch. L, Mounting plate for reed switch. M, Lamp. N, Lamp holder. O, Chassis. P, Lamp base. Q, Clamp for motor capacitor. R, Lamp stand. S, Shaft. T, Collar. U, Spring. V, Solar cell. W, Ammeter. X, Switches. Y, Dual banana connecter. Z, Quick disconnecters. a, Motor capacitor. b, Fuse holder. c, Battery holder clamp. d, Battery holder. e, Lamp shield. f, Hookup wire.

of Teflon, Plexiglas, and aluminum sheet metal is also required. About one-fourth inch of the lower front tabs of the vision-analyzer cabinet is removed to facilitate the entrance and exit of the chassis into the cabinet. The frame is attached to the chassis (figs. 4 and 5). The neutral-density disk and light chopper are cut from a sheet of Plexiglas painted flat black and assembled into the frame with the appropriate parts (figs. 6 and 7).

The reed switch (part of the oscilloscope trigger circuit) is assembled and wired as shown in figures 7 and 8 and attached to the frame. The solar cell for monitoring the light stimulus is fitted in its Teflon holder and attached to the rear of the frame. It is positioned into the edge of the chopped light beam going through the light pipe to the insect eye.

The filter holder (fig. 9) for the green filter (Kliegel No. 24) is attached to the inside of the cabinet. The light pipe is fabricated as shown in figure 10. The lamp filament should be parallel with the front of the cabinet and centered in the round opening of the frame to provide uniform illumination over the area of the light beam exiting the light pipe onto the insect eye. A 1½-inch-diameter hole is made in the rear of the cabinet after the frame and

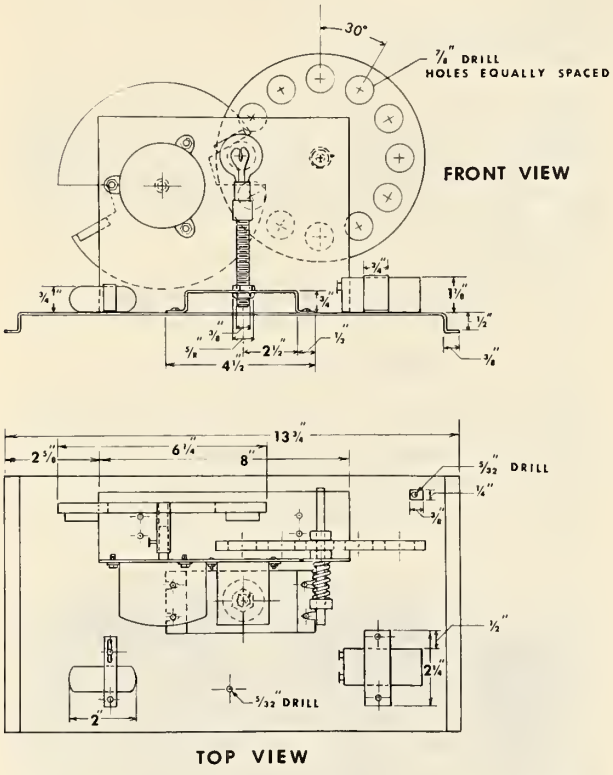

FIGURE 4.—Chassis of insect-vision analyzer with attached components.

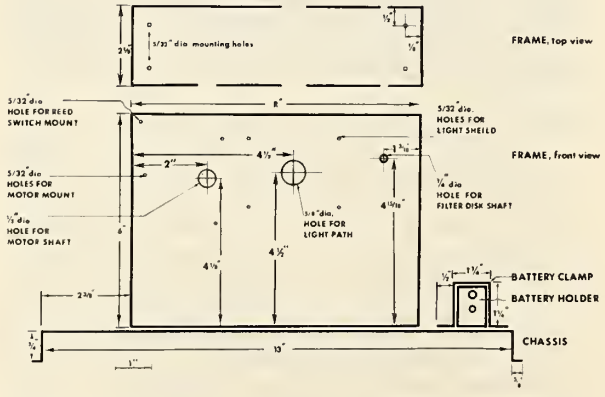

FIGURE 5.—Frame and chassis of insect-vision analyzer with battery-clamp assembly.

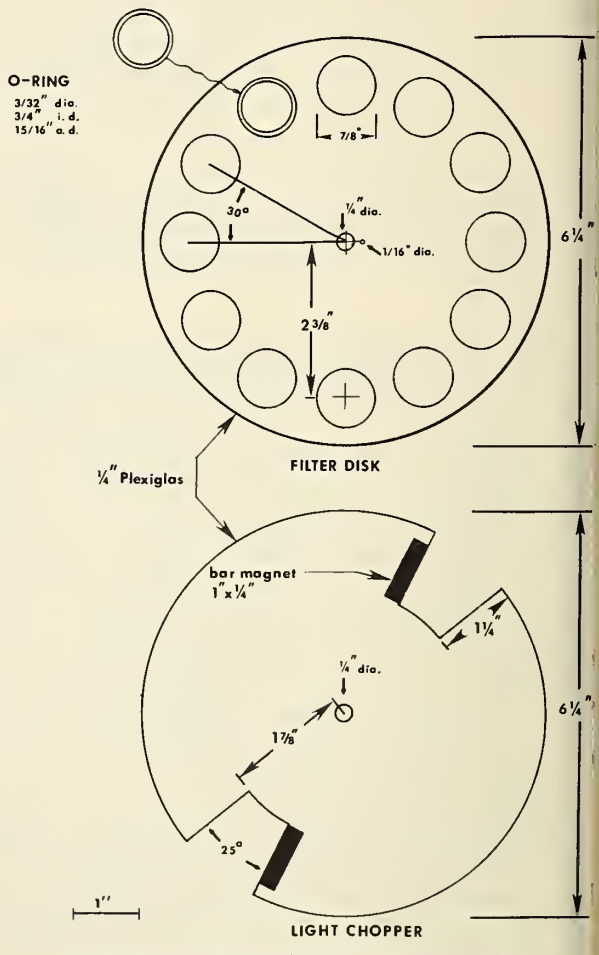

FIGURE 6.—Filter disk and light chopper.

light have been adjusted. The light pipe is attached to the rear wall of the cabinet with screws (fig. 10). The filter slots are for additional filters to reduce the light level (in combination with the filter disk) to maintain the criterion ERG response.

The electrical circuit for the motor, lamp, meter, and fuse is assembled as diagramed in figure 8; quick-disconnect clips are used to facilitate assembly and disassembly.

The slot permitting the neutral-density disk to extend above the hinged top to the cabinet is cut to match the disk after the entire chassis and frame have been assembled. The slot is one-half inch wide and 4 inches long. The neutral-density filters are cut to fit the 7/8-inch holes in this disk and are held in position by rubber 0-rings, one on each side of the filter. A centering mark is indicated on the top of the cabinet. The perimeter of the neutral-density disk is covered with an adhesive strip of white paper. The filter holes are individually alined for maximum passage of light from the lamp through the light pipe. As each hole is alined, the paper on the edge of the disk is marked to match the indicator mark on the cabinet corresponding to zero to 1.1 neutral density. Thus, the filter factor in front of the lamp is indicated on the disk edge by the mark in alinement with the indicator mark on the cabinet.

The assembled vision-analyzer unit (figs. 11–13) is alined with the Faraday cage (fig. 14) so that the center of the beam of light from the light tube is centered on the stand that holds the insect for testing. The stand and

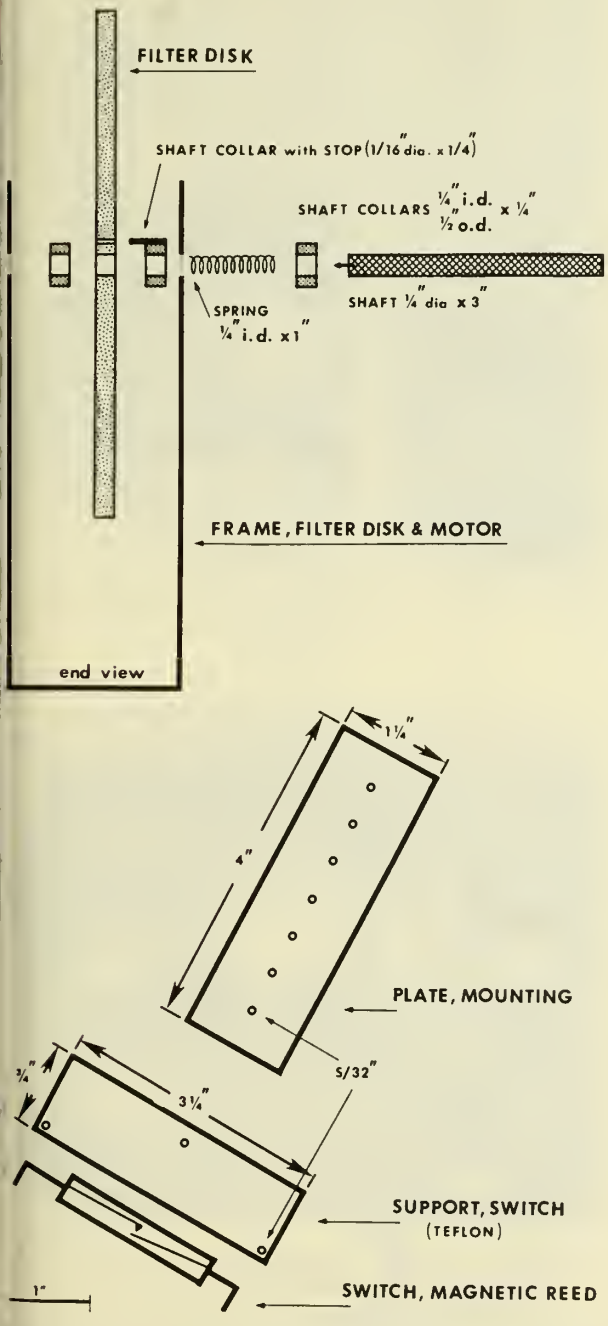

FIGURE 7.—Filter disk and reed-switch assembly. Front views (bottom) show components for making reed-switch support for attachment to frame.

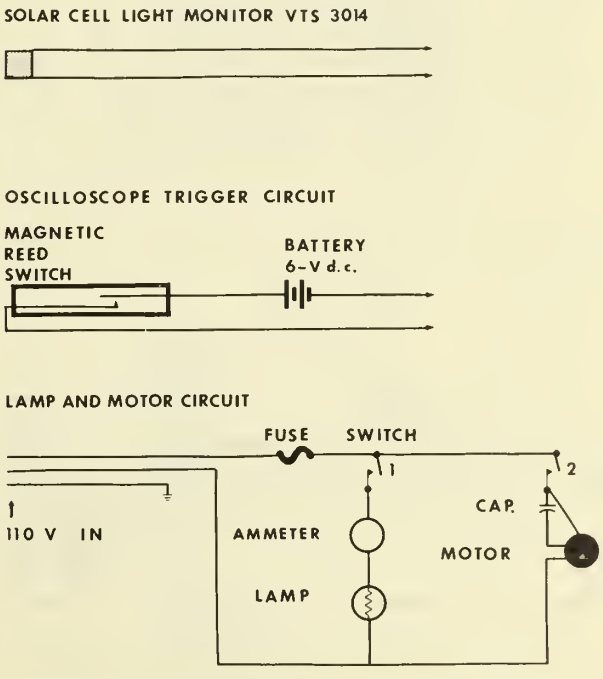

FIGURE 8.—Electrical circuits for insect-vision analyzer.

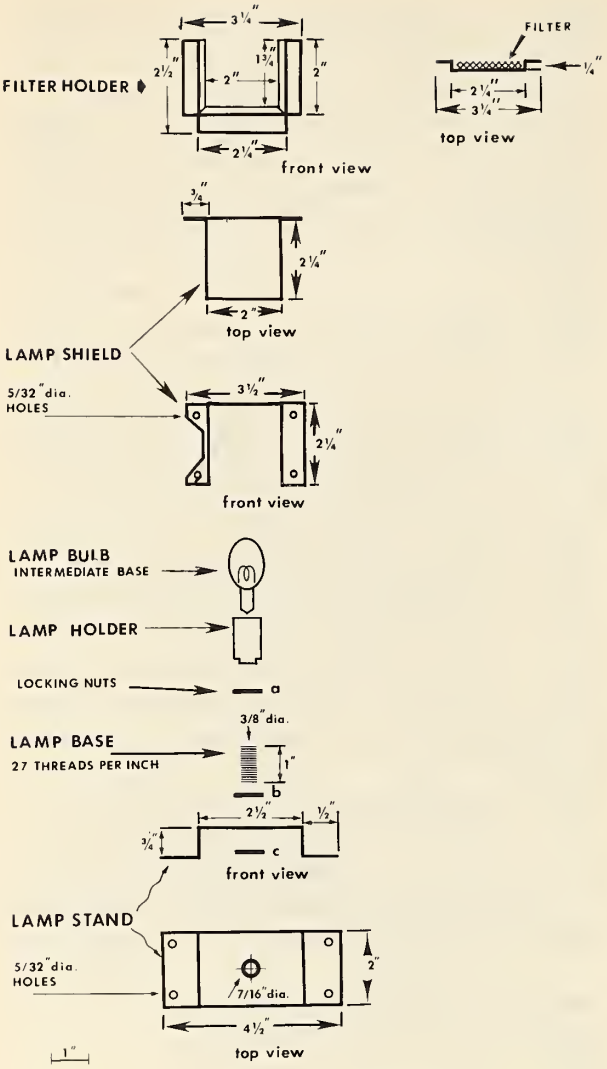

FIGURE 9.—Components for fabricating filter holder, lamp shield, lamp base, and stand.

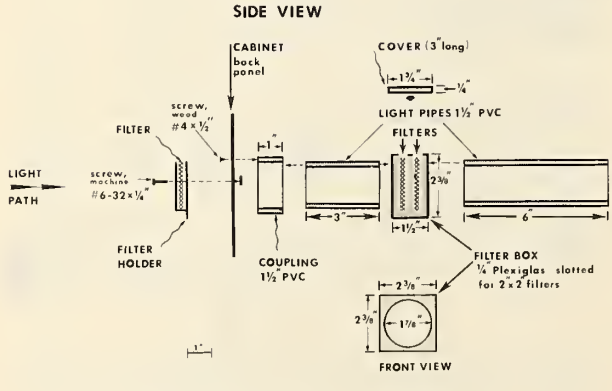

FIGURE 10.—Light pipe and filter assembly.

insect are centered in the visual field of the observer using the microscope.

To provide convenient viewing of the ERG response, the output of the vision-analyzer trigger circuit is connected to the external trigger circuit of the oscilloscope (fig. 15). Thus, the oscilloscope can be adjusted to provide a trace only when the light stimulus is applied to the insect eye. Also, corresponding parts of the response appear in the same relative position for successive pulses of light. The position of the reed relay is adjusted to synchronize the

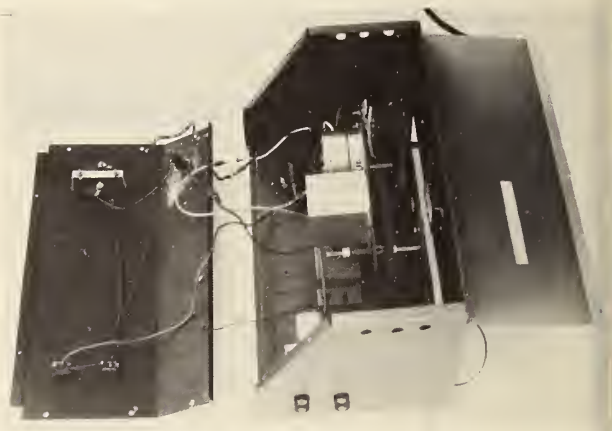

FIGURE 11.—Side view of interior of assembled vision analyzer.

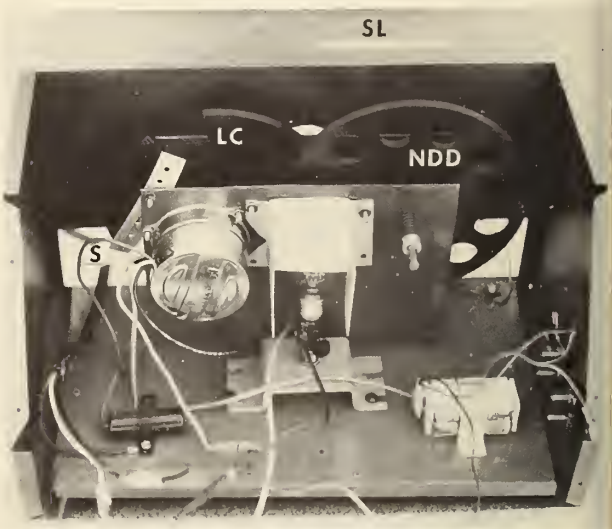

FIGURE 12.—Front view of interior of assembled visio analyzer. NDD, Neutral-density disk. LC, Ligh chopper. S, Magnetic reed switch. SL, Slit i cabinet cover to receive portion of neutral-densit disk extending above cabinet for thumb adjustmen to maintain criterion electroretinogram response.

FIGURE 13.—External view of assembled vision analyzer with attached light-conduction tube and filter holder.

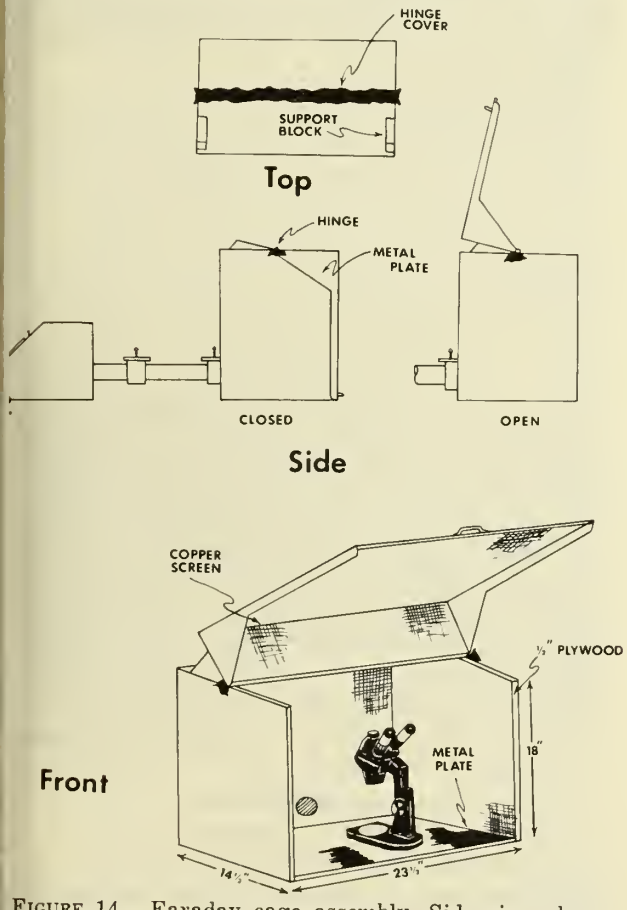

FIGURE 14.—Faraday cage assembly. Side view shows vision analyzer and light pipe connected to cage.

sweep of the light-monitor trace and the electroretinogram trace to begin simultaneously. The trace position on the oscilloscope may be adjusted with the horizontal-position control. After the fabrication and assembly have been completed, the system is ready for data collection.

Electrodes

Electrode etcher

An electrode etcher is used to make fine stainless-steel wires into electrodes suitable for penetrating the chitinized lens of the compound eye of the insect. The design of the circuit (fig. 16) is similar to that used by K. D. Roeder, Department of Biology, Tufts University, Medford, Mass. The components are mounted in a box 5¼ by 2¼ by 6¾ inches.

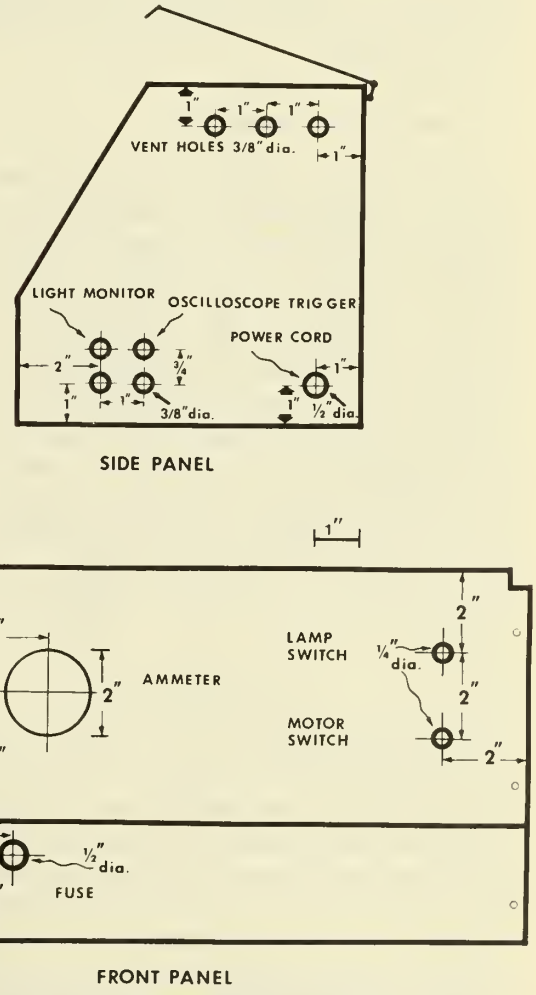

FIGURE 15.—Cabinet assembly of vision analyzer showing external connections.

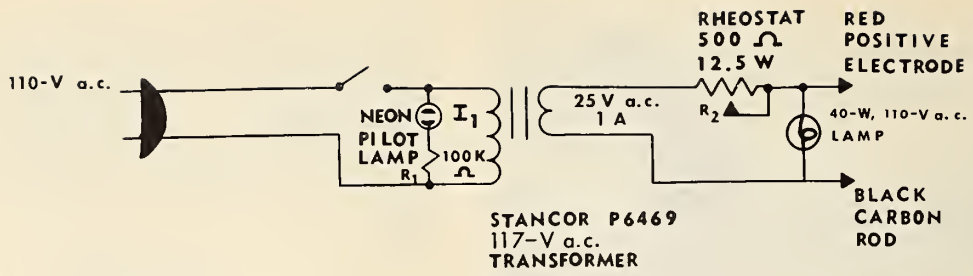

FIGURE 16.—Schematic of electrode etcher.

Etching solution

The etching solution is a combination of 34 ml of H_2SO_4, 42 ml of H_3PO_4, and 34 ml of water. The solution is poured into a petri dish to a depth of 1 cm. A thin layer (1 mm or less) of a light oil is floated on top of the etching solution to reduce spattering of minute droplets of acid produced when gas bubbles burst as the electrodes are being etched. *Caution should be used in handling and storing this corrosive etching solution.*

Electrode-making techniques

One of the clips from the etcher is connected to a carbon rod that has been placed in the etching solution. (The carbon rod can be obtained from the center of a C or D dry cell.) The other clip is used to hold the wire that is to be etched into an electrode. The stainless-steel wire cleaners from 26-gage hypodermic needles etch to sharp, strong points and have good conductivity characteristics when used in making electroretinograms. The 1½-inch wire is held in a vertical position and dipped into the etching solution. The etcher is turned on with the voltage control in the low position. The voltage is increased until the wire is rapidly etching without arcing as the tip is dipped into and removed from the solution. The dipping is repeated with a 2- or 3-second cycle, and the wire is submerged about 1 mm beneath the surface of the acid. This is the rapid etching phase, and the wire will start to taper to a point with the repeated dipping. In the next stage, the voltage is adjusted to medium to permit etching of the tip to a very sharp point with an angle of about 16° to 20°. After 2 to 3 minutes of etching the tip should be examined under the microscope at 50 to 100 power to make sure the electrode has been etched to a sharp point with the proper angle. Additional etchings may be necessary to properly shape and sharpen the electrode. The shape of the electrode is controlled by the length of time the wire remains in the etching solution, the rate of dipping the electrode into the solution, and the voltage applied. Reducing the voltage gives more precise control over the point sharpness. Very long, thin electrodes tend to bend when penetration of the insect eye is attempted. Practice in electrode etching and eye penetration for each species is required because of the varying shape and thickness of the lens of each insect. After the etching procedure, the electrodes are washed in a warm detergent to remove the oil residues and are rinsed in tap and distilled water.

The electrodes are joined to the leads from the biological amplifier in a 3 cm length of 1.5-mm-diameter glass or brass tubing filled with Wood's metal (a low-melting-point metal alloy, about 60° C). A butane cigar lighter is convenient for melting the metal to change electrodes.

Faraday Cage

The Faraday cage is a lightproof box lined with copper screen to provide an electrically quiet (grounded) area for insect preparation (fig. 14). The screen of the cage is grounded to a metal water pipe by a 12-gage copper wire and solder connections. The interior of the cage is painted with flat black paint to prevent reflections of the light stimulus. A ¼-inch-thick plate of copper, aluminum, or steel should be fastened securely to the floor of the cage to provide a smooth, firm base for the micromanipulators and microscope. The cage must be large enough to permit the use of the stereomicroscope and two micromanipulators for implanting the electrodes. The instrument arrangement inside the cage is shown in figure 17. The Faraday cage also provides a place for the

insect to adapt to the dark and a lightproof area for presenting the light stimuli.

Biological Amplifier

A biological amplifier (fig. 2) is used to match the input resistance and amplify the electrical signal detected by the electrodes in the eyes of the insects. The amplifier is positioned as close to the Faraday cage as is convenient (usually 2 to 4 inches) to reduce 60-cycle interference. A two-conductor shielded cable (26- to 28-gage) is connected from the amplifier input to the electrodes through a matching hole in the Faraday cage. The shield lead is connected to the ground of the amplifier and the screen-wire shield of the Faraday cage. The G–1 lead is connected to the electrode positioned in the illuminated eye, and the G–2 lead is connected to the electrode in the indifferent (nonilluminated) eye through a terminal block. Usually it is more convenient to use fine, 28- to 30-gage, silver wire (5 cm long) to make the connections from the terminal block to the electrode holders. These bare wires should be parallel to one another to prevent uneven pickup of stray 60-cycle interference. Also, the bare wires must not touch any metal surface. An amplification factor of 1,000 is used with filter settings of 3 Hz and 0.1 kHz.

Oscilloscope

Although nonstorage oscilloscopes with long-persistence phosphors are satisfactory for the experienced operator, the dual-beam storage oscilloscope is highly preferred. The storage oscilloscope (listed in appendix A) is more convenient for the beginner because it can be adjusted so that the trigger output of the vision analyzer automatically triggers the beam on a single-sweep storage setting of the oscilloscope, which provides a display of the light stimulus monitor (solar cell) on one beam and the eye response on the other beam. The operator can carefully measure the "on" response and adjust the neutral-density filtering to maintain the criterion response.

Stereomicroscope

The stereomicroscope inside the Faraday cage (fig. 17) should provide magnifications of 10 to 100 times to facilitate electrode positioning

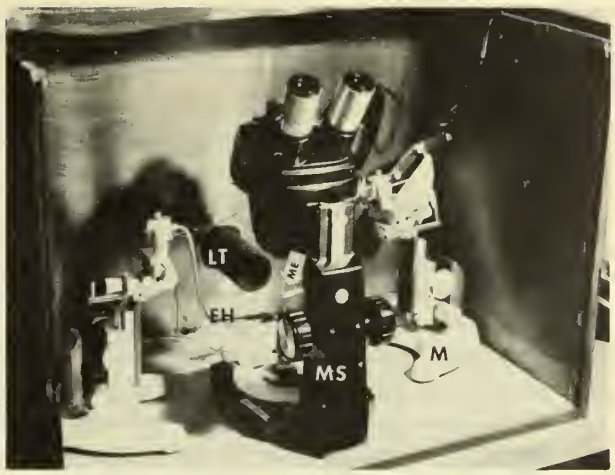

FIGURE 17.—Interior view of Faraday cage. M, Micromanipulators. EH, Electrode holders. ME, Microelectrodes. LT, Light-connecting tube. MS, Microscope.

and insertion into the eye of the insect. Much time is saved in setting up insect-eye preparations by fastening the base of the microscope to the floor of the cage. The proper location is determined after the microscope is set at high magnification and the insect is centered in the visual field of the microscope and in the light beam from the insect-vision analyzer. The next preparation is simply placed under the microscope on low magnification, centered, checked at high magnification, and recentered if necessary. Thereafter, the eye is automatically centered in the light beam and ready for testing. It is most convenient to work under low magnification while bringing the electrodes into position over the eye, but the insertion of the electrodes into the eye is accomplished under high magnification to obtain the most precise control of electrode movement and to determine the proper depth of electrode penetration into the eye.

Micromanipulators

A pair of micromanipulators (listed in appendix A) are used to insert the electrodes into the compound eyes of insects for recording the electroretinogram (fig. 17). A three dimensional rack-and-pinion and screw-drive unit has proved most satisfactory. It has calibrated knobs and pointers to indicate the distance traveled. An arm is fabricated from ¼-inch stainless-steel tubing and fitted with an alligator clip for holding the electrodes. The jaws

of the clips are covered with heat-shrink tubing to insulate the micromanipulator from the electrode.

PROCEDURES

Mounting the Insect for Testing

The insect is mounted on a stand that has a smooth metal base about 1½ inches tall with a 1-inch ball of tackiwax on top. A galvanized pipe reducer (½ to ¾ inch with the larger end sanded smooth and level) makes a convenient stand. The tackiwax ball can be shaped to receive the insect, and it holds miniature staples well. The staples are made by bending pieces of paper staples or used electrode wires into sizes to fit the insect. The legs of the insect may be removed with fine-pointed scissors. The insect is held in place on the stand with the staples over the wings or body or both, depending on the insect. With 4-inch hemostats or No. 5 jeweler's forceps, a tiny staple is placed behind the head to immobilize it. With some insects it is necessary to add some support from the ventral side of the head by pushing the tackiwax up against the mouthparts (fig. 18). Care must be exercised to avoid blocking the light to the eye or injuring the eye when setting up the preparation. It is best to work under a stereomicroscope to prevent accidental injury to the insect. The insect should now be securely mounted on the tackiwax ball, with one eye positioned in the light beam from the insect-vision analyzer and the other eye entirely in the shadow of the illuminated eye.

Electrode Placement

The recording electrode (G–1) should be carefully inserted 20 to 50 μm just beneath the lens of the illuminated eye, with the tip of the electrode close to the rhabdom (the photoreceptor part of the ommatidium) to minimize receptor damage (fig. 1). The micromanipulator is used to insert the electrode into the eye. A similar or indifferent electrode (G–2) is inserted into the nonilluminated eye; it detects only the resting potential of the eye. The dorsal quadrant at the midline between the anterior and posterior margins of the eye has proved most satisfactory for electrode insertion (fig. 18). When the electrodes are removed from the eye after a test, they should be rinsed with a jet of distilled water to remove any cellular debris that may have adhered to the electrodes. Sometimes it is necessary to wipe the electrodes gently with a wet, soft paper tissue with a downward movement toward the etched tips. Care must be exercised in cleaning electrodes to avoid damaging the tips. Placement of the microelectrode into most insect eyes causes so little injury that an insect released after testing displays normal behavior patterns if the legs and other body parts are left intact.

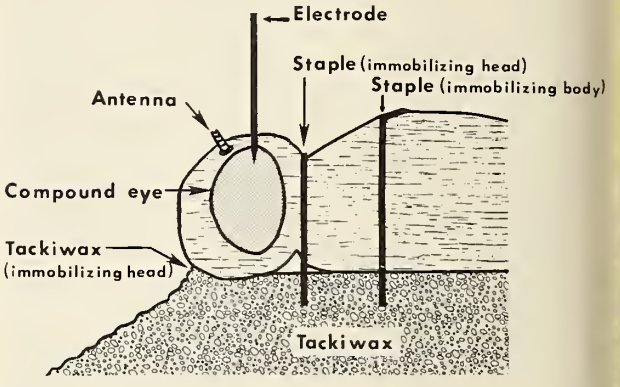

FIGURE 18.—Side view of compound eye showing typical site for insertion of electrode.

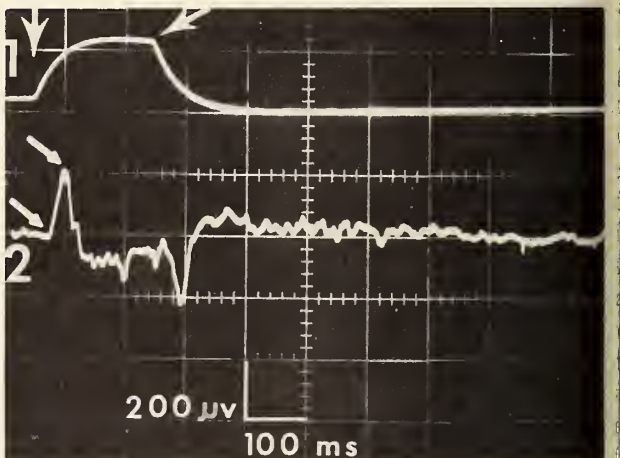

FIGURE 19.—Sample electroretinogram. Sweep 1, First arrow indicates beginning of light stimulus ("on" response), and second arrow indicates end of stimulus. Sweep 2, Lower arrow indicates baseline of the electrical potential of nonilluminated eye compared to reference eye, and upper arrow indicates top of "on" component recorded from illuminated eye as compared to reference or nonilluminated eye. (The 1-cm upward deflection of oscilloscope trace is a 200 μV criterion response.)

Electroretinogram Technique

Verification of correct positioning of the electrodes and equipment operation is checked by using the full intensity of the light from the insect-vision analyzer. The operation check, the dark adaptation, and the visual sensitivity tests are made with the microscope light "off" and the cover for the Faraday cage closed. When the typical electroretinogram response has been obtained, the insect eye is allowed to adapt in the dark to its most sensitive state, which requires 20 to 45 minutes, depending on the insect species; day-flying insects require shorter periods. The light chopper (fig. 6) of the vision analyzer generates short pulses of light (about 250 ms) that are beamed onto the eye. All the insects tested have responded well to the green (500 to 530 nm) light from the vision analyzer. When the insect eye is stimulated with light, electrical changes occur in the eye that can be detected by the electrodes, amplified by the biological amplifier, and displayed on the oscilloscope for measurement. A neutral-density filter is brought into the light path to maintain the intensity of the light to the eye at a level that elicits a criterion (200 μV) response (fig. 19). This light level will not cause receptor adaptation when repeated stimuli are presented.

A small bar magnet attached to the leading edge of the light-chopper slots closes the magnetic reed switch. The closed switch sends a 6-V d.c. pulse to the trigger circuit of the oscilloscope to trigger the beam sweep just before the light beam strikes the insect eye (fig. 8). This arrangement causes the sensory responses of the insect to be displayed on one beam of the oscilloscope and synchronizes the sweep of the two beams of the oscilloscope. The light-stimulus monitor from the solar cell is displayed on the other beam. The solar cell is used to indicate when the stimulus is "on" and "off" and facilitates the identification of the "on" receptor response of the insect eye. The "on" response (fig. 19) is the leading negative depolarization of the receptor cells when they are stimulated with light.

In the recording of the ERG data, the neutral-density filters are used to adjust the light level to give a 200 μV "criterion" response at the insect eye. The vertical amplifier of the oscilloscope is so calibrated that 200 μV before amplification a 1-cm vertical deflection occurs after the 1,000-power amplification by the biological amplifier and the appropriate amplification on the vertical amplifier of the oscilloscope. Therefore, a 1-cm "on" response (criterion response) is detected by the electrodes in the insect eye when the light stimulus strikes the eye.

The filter wheel is adjusted to maintain the 1-cm deflection of the vertical amplifier, and the neutral-density factor is recorded along with other related data onto data sheets similar to that in appendix C. I have found that testing 5 to 10 insects from one group is usually a sufficient number for comparison with data from other groups. The insects successfully tested with the ERG are listed in appendix D.

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APPENDIX A.—INSTRUMENTS AND MATERIALS REQUIRED TO RECORD THE ELECTRORETINOGRAM

Amplifier, Grass, model P–15 (cost, about $250 in 1977).
Electrode etcher.
Electrode holder, shaped from ¼-inch-diameter stainless-steel tubing to fit micromanipulator and receive alligator clip for holding microelectrode connecters.
Faraday cage.
Filters (0.1 to 1.0 neutral-density), Kodak neutral-density gelatin.
Filter, Kliegel No. 24, peak-band pass at 515 nm.
Forceps, No. 5 jeweler's.
Hemostat, curved or straight, 4 inches, to insert wire staples into tackiwax.
Micromanipulators (2 each), Brinkman; BL movement (Z) #06956; base and pillar #069077–09; H S movement with scales and pointers; clamp E and inclination joint X–Y #0640–12–01 (about $311 in 1976).
Oscilloscope, storage, Tektronix 5103/D11; amplifier #5A18N; time base #5B10N; and blank panel #016–0195–00 (about $1,750 in 1976).
Stereomicroscope, 10- to 100-power.
Tackiwax, Cenco Co.
Scissors, fine points, to remove insect legs and antennae.
Insect-vision analyzer.
Wire, stainless- steel; No. 26 needle cleaners for making electrodes.

APPENDIX B.—MATERIALS REQUIRED TO CONSTRUCT THE INSECT-VISION ANALYZER

Battery, size AA, 4 each.
Cabinet, sloping-panel, Bud type C 1894, 8- by 14⅛- by 8-inch.
Clamp, battery, shaped from 4¾- by ¾-inch aluminum (0.05 inch thick).
Clamp, for capacitor, 3- by ¾-inch aluminum (0.05 inch thick).
Clip connecter for battery holder.
Connecters, quick-disconnect, 6 each male, 6 each female.
Cord, power, 6-foot, size 3–14.
Disk, filter, ¼-inch-thick Plexiglas, 6¼-inch diameter.
Filter, Kliegel cinemoid No. 24, 2- by 2-inch.
Filter, neutral-density (0.1 to 1.0), Kodak gelatin or equivalent.
Grommet for power cord.
Holder, battery, for size 4 AA with terminals.
Holder, filter, made of ¼-inch Plexiglas to receive three 2- by 2-inch filters.
Holder, filter, shaped from aluminum (0.05 inch thick), 6- by ¾-inch.
Holder, lamp, intermediate screw base with ⅜-inch threaded connecter.
Lamp, tungsten, 40-W, 120-V, intermediate screw base.
Lamp base support, shaped from 6- by 1⅞-inch aluminum (0.05 inch thick).
Light chopper, ¼-inch Plexiglas, 6-inch diameter, ¼-inch center hole with brass bushing fitted and tapped for screw to attach to motor shaft.
Light conducting pipe, 1½-inch PVC, 2-foot with coupling.
Magnet, Hamlin H–32–605, 2 each.
Meter, AC amp, 0–500 mA.
Motor, 10-r/min, Hurst model DA and capacitor.
Motor support, shaped from 14- by 8-inch aluminum (0.09 inch thick).
O-ring, #7693, ¾-inch i.d., $\frac{15}{16}$-inch o.d., 3/32-inch diameter, 24 each.
Panel, base, 14½- by 7¾-inch aluminum (0.09 inch thick).
Post, dual binding, 2 each for double banana plug.
Screws, machine, 6/32-inch diameter, ½-inch long, 24 each.
Shaft collar, ¼-inch, 3 inches long.
Shaft collar, ¼-inch, 3 each.
Solar cell, ⅛- by ¼-inch, VTS 3014.
Spring, compression, 1½ inches long, ¼-inch inside diameter.
Support, Teflon, for switch, magnetic 1- by 2- by ⅛-inch.
Support, Teflon, for solar cell, 1- by ½- by ⅛-inch.
Switch, SPST, 120-V, 2 each.

Switch, magnetic reed, Hamlin DRR–5425.
Tubing, heat shrink, ⅛-inch diameter, 6 inches long; ¼-inch diameter, 6 inches long.
Wire, hookup, 5-foot.

APPENDIX C.–SAMPLE DATA SHEET FOR RECORDING SPECIFIC DATA ON INSECT AND FILTER FACTOR REQUIRED FOR A CRITERION RESPONSE

No._____

Date_____ Species_____ Sex_____

Source— Field_____ Lab_____ Diet_____

Age _____

Hour set up_____ Hour tested_____ Response: N.D._____
(neutral density)

Electrode shape_____

Problems_____

Total neutral-density factor for insects in test group:

Mean_____

Lamp mamp_____ Range_____

Tested by_____ _____

APPENDIX D.–INSECTS SUCCESSFULLY TESTED WITH THE ELECTRORETINOGRAM TECHNIQUE

Common name	Scientific name
Apple maggot fly	*Rhagoletis pomonella* (Walsh).
Bollworm moth	*Heliothis zea* (Boddie).
Boll weevil	*Anthonomus grandis* Boheman.
Caribbean fruit fly	*Anastrepha suspensa* (Loew).
Mediterranean fruit fly	*Ceratitis capitata* (Wiedemann).
Olive fly	*Dacus oleae* (Gmelin).
Screwworm fly	*Cochliomyia hominivorax* (Coquerel).
Stable fly	*Stomoxys calcitrans* (Linnaeus).
Tobacco budworm moth	*Heliothis virescens* (Fabricius).
Tsetse fly	*Glossina morsitans morsitans* Westwood.
Do	*Glossina palpalis palpalis* (Robineau-Desvoidy).

* 1977-GPO-1750-S/771-040/19

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